COLLABORATIVE STUDIES ON THE DETERMINATION OF AFLATOXINS IN PEANUT PRODUCTS IN FRANCE

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ABSTRACT

In order to develop a method for the determination of aflatoxins in peanuts, peanut products, cereals and cereal products (major components of feed) we made a study of some existing procedures (CB, TPI, BF, Celite, Pons).

Collaborative studies using these procedures were undertaken. The CB procedure was selected on account of the quality of the obtained extract, the low level of background interference and the relative precision and simplicity of the required equipment.

Taking into account the very frequently high level of aflatoxin B_1 (around 1 ppm) in peanut and the quality of silica gel and chloroform available in France, some minor changes have been incorporated into the CB procedure: the chloroform used for the column is washed and dried before use, the volume of elution is increased and an ether–methanol–water mixture (96:3:1) is used as a developing system.

The natural world-wide occurrence of aflatoxins in different agricultural commodities justifies the extensive research by various investigators on the development of suitable analytical methods for their detection and determination. The problem of aflatoxin contamination in peanuts and cereals assumed great importance in this country. Commercial peanuts and cereals very frequently show the presence of 1 mg kg^{-1} and 5 to 50 µg kg^{-1} of aflatoxin B_1 respectively. Furthermore, discussions have now opened between the members of EEC in order to determine a tolerance of aflatoxins in feed (which, in France, is composed mainly of peanut meal and corn). It would be desirable to totally eliminate aflatoxins in food and feed but it still remains a utopia. Should we resign ourselves to accept the presence of a certain quantity of aflatoxins in our food? Just now the choice of tolerance is based on an empirical estimation to give protection against hazard on the one hand, and on the aflatoxin quantities really present as natural contaminants in the products on the other.

For all these reasons it becomes necessary to elaborate methods for aflatoxin determination in the peanut products and cereals. This paper presents the results of collaborative studies conducted in France and gives the reason for the choice of the analysis method, that we have adopted in France, for the determination of aflatoxins in peanut meal and cereals.

In order to develop a method applicable in routine analysis to the determination of aflatoxins in peanut products and cereals, five existing

techniques were first selected on the basis of different concepts which have been summarized recently by Liem and Beljaar[1] with respect to the preparation of the aflatoxin extracts: (*i*) pre-defatting of the sample followed by solvent extraction with Soxhlet apparatus; TPI[2] procedure—(*ii*) direct extraction from the sample with a water-miscible solvent (70 per cent acetone); Pons *et al.*[3] procedure—(*iii*) simultaneous defatting and extraction with a two-phase-solvent system; Celite[4] and BF[5] procedures—(*iv*) simultaneous defatting and extraction with one solvent; CB[6] procedure.

The choice of the procedure was made after a consideration of the following factors: simplicity, rapidity, quality of the obtained extract with low level of background interferences, reproducibility (precision) and possible application to peanut products and cereals.

The first collaborative study was organized in 1969 by the ITERG (Technical Institute of Fat Products in Paris); the TPI procedure was compared with the rapid method of Lee[7] (*Table 1*).

Table 1. Collaborative results for analysis of aflatoxin B_1 ($\mu g\ kg^{-1}$) in peanut products by TPI and Lee methods (11 laboratories)

Summary	TPI method		Lee method	
	Lower observable fluorescence–extinction	Comparison to standard of aflatoxin B_1	Lower observable fluorescence extinction	Comparison to standard of aflatoxin B_1
Mean ($\mu g\ kg^{-1}$)	450	1020	545	1085
Standard deviation	285	605	480	445
Coefficient of variation (%)	63	59	88	41

It was concluded that the TPI method is not only tedious, but appears to give a loss in aflatoxin content which could be explained by a number of manipulations. This procedure does not appear to be applicable in routine analysis. As shown in *Table 1*, the estimation of aflatoxins by fluorescence–extinction has to be eliminated with regard to the coefficient of variation.

A second collaborative study was organized in 1970 during a training session on the analytical procedures for aflatoxin assays; 12 laboratories collaborated, 4 techniques were examined (Pons *et al.*, Celite, BF, CB methods) and 2 types of products were used, peanut meal and wheat flour (*Table 2*).

Table 2. Comparison of aflatoxin B_1 ($\mu g\ kg^{-1}$) determination in peanut meal and wheat flour by 4 analytical procedures

Summary	Pons *et al.*		Celite		BF		CB	
	Wheat flour	Peanut meal	Wheat flour	Peanut meal	Wheat flour	Peanut meal	Wheat flour	Peanut meal
Mean ($\mu g\ kg^{-1}$)	23	542	21	510	10	430	32	570
Standard deviation	8.58	216.8	11.02	180.59	4.51	175.05	9.69	153.9
Coefficient of variation (%)	37.30	40	52.47	35.40	45.1	40.70	30.28	27

The results of this study show that the CB procedure is more precise than the others. The simplicity of required equipment, the low level of background interferences and the possible application to peanut products and cereals should result in a preference for the CB procedure for routine determination of aflatoxins in peanut products.

Taking into account the very frequently high level of aflatoxin B_1 in peanut, higher than (1 mg kg^{-1}) and the quality of chloroform and silica gel available in this country some minor changes have been incorporated into the CB procedure.

(*i*) Chloroform used for the column is washed and dried before use. We found that a certain quality of chloroform, probably one stabilized with an alcohol content higher than 1 per cent, eluted aflatoxin at the beginning when the extract was deposited on the column.

(*ii*) The elution volume is increased from 150 ml to 250 ml.

(*iii*) Ether–methanol–water mixture (96:3:1) is used as a developing system. This solvent gives a better separation of the four aflatoxins than the classical solvents acetone–$CHCl_3$ or $CHCl_3$–methanol.

Two other collaborative studies were organized with the help of ITERG using the slightly modified CB procedure. They were concerned with the determination of aflatoxins in peanuts and peanut products (*Table 3*).

Table 3. Collaborative results for analysis of aflatoxin B_1 (μg kg^{-1}) in peanuts and peanut meal by CB modified method (11 laboratories)

Summary	Peanut	Peanut meal
Mean (μg kg^{-1})	749.7	485
Standard deviation	134.68	65
Coefficient of variation (%)	17.9*	13

* For this assay if the values of two of the collaborators are discarded the coefficient of variation is 11.43 per cent.

The results are excellent with regard to the coefficient of variation. The evaluation of aflatoxin B_1 was based on a comparison of the fluorescent intensities of spots of sample with those of standard spots, the instrumental evaluation of the t.l.c. plates could probably improve the coefficient of variation. In this respect a study of the reproducibility (variation between plates) and the repeatability (variation in the same plate) of aflatoxin B_1 evaluation by reflectance fluorodensitometry was undertaken[8]. Vitatron TLD 100 chromatogram scanner was used; this system is similar to Nester Faust Uniscau 900 described by Pons[9].

The results show that our data, at least those concerning the repeatability expressed as reproducibility by Pons, are essentially in agreement and that it was advantageous with respect to precision to use instrumental evaluation.

To conclude, the CB procedure for extraction and separation is suitable for aflatoxin B_1 in peanut meals and cereals. This procedure is largely accepted in France and most probably will be adopted in the other countries of the EEC.

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